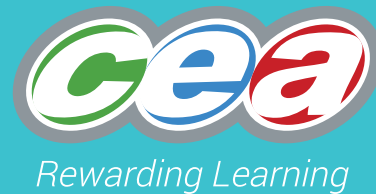


GCSE



CCEA GCSE TEACHER GUIDANCE

# Biology Practical Manual

## Unit 3: Practical Skills

- 2.3 Investigate the effect of chemicals or antibiotic discs on the growth of bacteria



## **Practical 2.3**

### **Investigate the effect of different chemicals or antibiotic discs on the growth of bacteria**

#### **Introduction**

Students could make their own agar or teacher could demonstrate how to make one and provide plates freshly prepared by technical staff.

There is scope for developing or assessing a range of experimental and investigative skills (including students' ability to make a risk assessment). The basis of this practical is to assess the effectiveness of

- different brands of disinfectant products
- the effect of changing concentration of disinfectant products
- the effect of different antibiotics

#### **Apparatus and Chemicals**

For the class – set up by technician/teacher:

- microbial broth
- sterile Petri dishes, 1 or 2 per group
- sterile forceps, 1 or 2 per group
- disinfectant solution, 1 discard beaker per group

For each group of students:

- Bunsen burner
- nutrient agar – in a sterile McCartney/Universal bottle, 1
- materials with anti-microbial action, according to the students' hypotheses
- paper discs, Whatman antibiotic assay paper discs, or new filter/chromatography paper cut with a hole punch then sterilised by autoclaving, 4-8
- sterile forceps
- adhesive tape
- marker pen

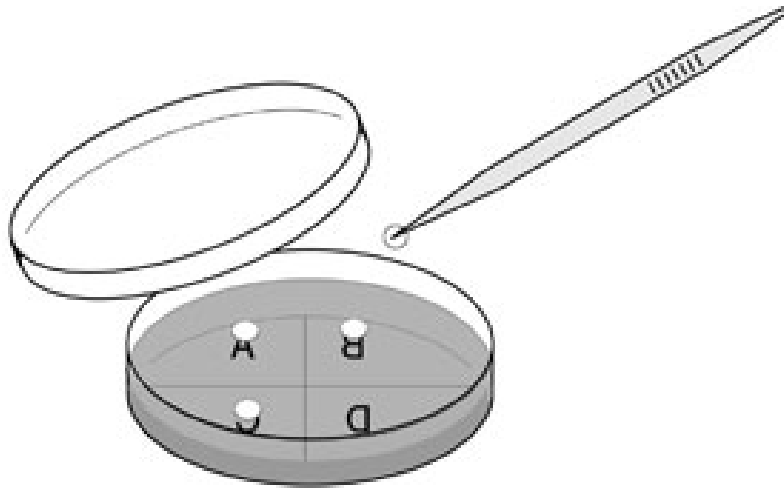
## **Risk Assessment**

- Use microbes from the 'safe micro-organisms' list provided CLEAPSS (Table 15.2 in Section 15 of the Laboratory Handbook). These microbes present the minimum risk given good practice. Recognized educational suppliers should provide safe strains. The *E. coli* K12 strain is not thought to have any harmful effects, and is preferable because of its more rapid and consistent growth.
- Ensure that no members of the group are debilitated or taking immuno-specific medication, as this may increase their risk of infection by the bacteria used.
- When using any commercial products, refer to manufacturers' guidelines, avoid contact with eyes, and limit skin contact.
- Take particular care that ethanol used to sterilize instruments is kept away from lit Bunsen burners. See CLEAPSS Hazcard: ethanol is HIGHLY FLAMMABLE and IDA is HARMFUL.

## **Procedure**

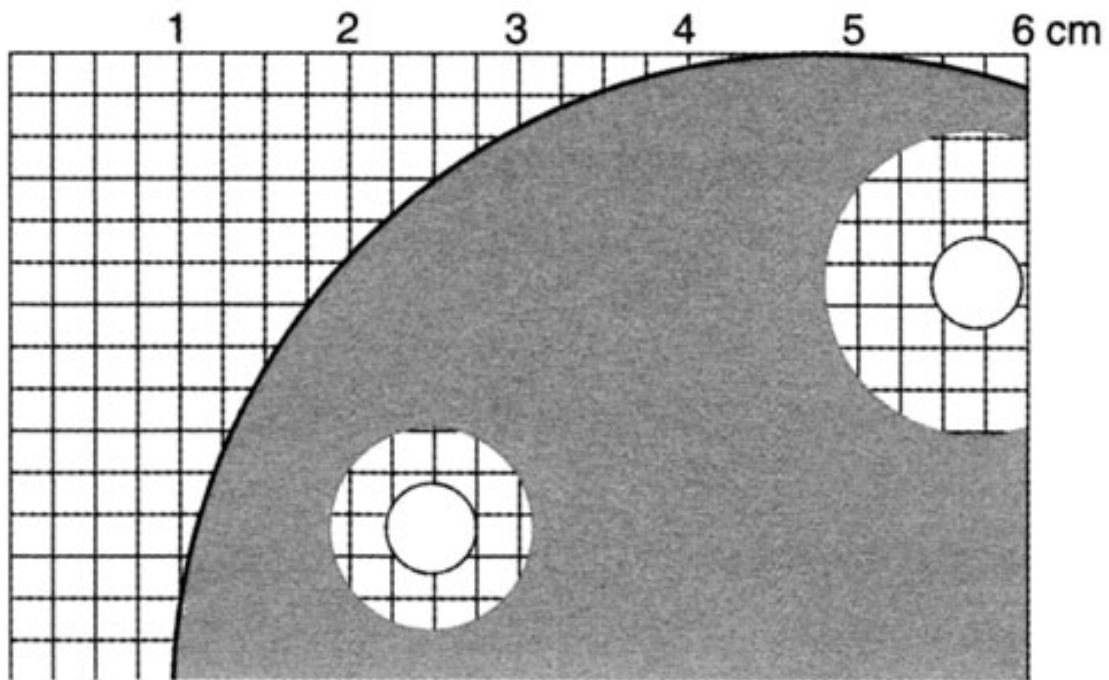
Students will need to observe basic hygiene rules and follow aseptic techniques. They need to be aware of what to do with spills and how to dispose of cultures and contaminated equipment.

Your risk assessment should take into account your students' behaviour and all plates should be stopped by treatment with methanol if students cannot be trusted to leave the plates closed, and not to remove them from the laboratory.



## Method

1. Each group will need to prepare a pour plate seeded with bacteria, or have one provided.
2. Make up the solutions or collect samples to test from the range of disinfectant solutions provided.
3. Prepare paper discs for each disinfectant solution used by dipping a small paper disc into the disinfectant or antibiotic solution using sterile forceps, taking care not to mix the discs up.
4. 4 discs per dish work well as shown above. A disc dipped in sterile water should be used as a control.
5. When the agar has set, turn the dish upside down. Divide the base into four sections by drawing a cross with the marker pen. Label the sections A, B, C, D.
6. Using sterile forceps, place a paper disc in each section; record the treatment of each disc. Flame forceps (or place in disinfectant) if they contact the surface of the agar and have been contaminated.
7. Label the agar plate with your name and date. Tape the lid but do not seal. Incubate inverted for 2-3 days at 20-25 °C.
8. Observe the plates without opening them.
9. Make any measurements that will help you to compare the anti-microbial properties of the different substances. A piece of squared paper under the agar plate might be helpful here. This is placed under the Petri-dish and the number of exposed whole squares are counted for each area and recorded in an appropriate table.



### **Conclusion**

The greater the area affected around the paper disc, the more anti-microbial the substance.

### **Alternative Investigations**

This experiment could be modified to investigate the effect of a range of anti-microbial substances such as:

- mouthwashes
- essential oils such as tea-tree oil or mint oil (to tie in with section 2.6.6 of GCSE Biology)
- spices such as cinnamon or cloves